THE TRYPANOCIDAL EFFECT OF PHENYLGLYCINE AMIDO ARSENATE OF SODIUM ON *T. BRUCEI* IN RATS AND *T. RHODESIENSE* IN MICE

BY

S. ADLER, M.B.

(*Received for publication* 23 *November*, 1921)

This drug* is a white amorphous powder readily soluble in distilled water, and yielding in 5 and 10 per cent. concentration a perfectly clear, colourless solution. On standing, however, for some days, a yellow colour develops, whether the solution is kept exposed to light or in the dark. A 5 per cent. solution when exposed to light became yellow in seven days; when kept in the dark, in six days. Daily sterilizing for ten minutes did not prevent the development of the yellow colour, but rather accelerated it.

Rats. Minimum lethal dose. In Table I are set out the experiments performed in order to ascertain the minimum lethal dose for rats; in all cases the drug was used in a freshly prepared solution of 5 or 10 per cent., and was injected intraperitoneally; it produced no irritation. Healthy and infected animals were used in this experiment.

It will be seen from the table that no dose was toxic to the animals injected until the amount of 1·2 gms. per kilo was attained.

Relationship of Toxicity to age and change of colour of solutions.

The number of experiments bearing on this point is rather limited, but serves to show that the toxicity increases on standing, *e.g.*, although the minimum lethal dose of freshly prepared solution for rats proved to be 1·2 gms. per kilo body weight, an animal which was injected with 0·18 gms. per kilo of a twenty-four hour old solution followed on the next day by a dose of 0·36 gm. per kilo of a forty-eight hour old solution, died after severe symptoms of poisoning on the sixth day after the second injection. In the

* Kindly put at our disposal by Messrs. May & Baker, Ltd., Battersea, London.

TABLE I.

Showing the minimal lethal dose for rats.

No. of Experiment	Dose in gms., per kilo	Infected or healthy	Remarks
1	0·33	Healthy	No toxic effects
2	0·46	Healthy	No toxic effects
3	0·43	Healthy	No toxic effects
4	0·67	Infected	No toxic effects
5	0·68	Infected	No toxic effects
6	0·7	Infected	No toxic effects
7	0·71	Healthy	No toxic effects
8	0·75	Infected	No toxic effects
9	0·82	Infected	No toxic effects
10	0·90	Infected	No toxic effects
11	1·0	Healthy	No toxic effects
12	1·1	Healthy	No toxic effects
13	1·2	Infected	Animal died in 22 hours
14	1·4	Healthy	Died on 4th day

above solution, although highly toxic, no change in colour was evident. Further experiments proved that in still older solutions the toxicity diminished, *e.g.*, on the eleventh day, when the solution was deeply yellow, three animals were each given a dose, approximately equal to the combined doses given above, without producing any symptoms. The first of these three animals was injected with a solution which had been kept in the daylight and boiled each day for ten minutes, the loss from evaporation being made up by addition of distilled water to the original volume before injection. The second was injected with a solution which was unboiled and kept in daylight; the third with solution unboiled and kept in the dark.

The increase in toxicity which was evident in the forty-eight hour old solution, was accompanied by a definite increase in trypanocidal power, for after injection of 0·36 gm. per kilo, trypanosomes disappeared from the peripheral blood within twenty hours, and were absent from the blood until the time of death. Examination for trypanosomes of the organs by smears proved negative, as did also the injection of emulsion of organs and blood into healthy rats. A similar sterilizing effect could only be produced by much larger doses of the freshly prepared drug, *e.g.*, a dose of 0·67 gms. per kilo in freshly prepared solution injected into a rat at approximately the same stage of infection as the previous one, only caused the disappearance of the parasites from the peripheral blood in forty-eight hours, and did not prevent their re-appearance twenty-four hours later.

Toxic effects of freshly prepared solutions.

No toxic effect was observed below the dose of 1·2 gms. per kilo; a heavily infected animal died within twenty-four hours of receiving this dose. The signs of poisoning noted before death were blindness and refusal to take food. No gross haemorrhages were found after death, but the whole intestinal tract showed numerous minute haemorrhages. No trypanosomes were found in the peripheral blood, either immediately before or after death.

Toxic effects of old solutions.

Toxic effects were noted after a dose of 0·36 gm. per kilo, but not until four days had elapsed. On the fifth day the

animal appeared ill, lying curled up, breathing irregularly and spasmodically, it refused food, was blind, and staggered in its gait when moved. Haemorrhages were observed from the conjunctiva, anus and urethra. Post-mortem, the whole intestinal tract was found to be haemorrhagic, the liver was enlarged and soft, and showed yellow mottling; the kidneys were very soft, but not enlarged nor dark in colour; the spleen showed yellowish patches.

Minimum curative dose.

In Table II are set out the experiments performed in order to demonstrate the minimum curative dose in rats.

Table II.

Shewing effect on rats infected with *T. brucei*.

Experiment	Trypanosomes per field. Ocular 4, Obj. $\frac{1}{8}$	Dose in gms., per kilo	Day of disappearance of trypanosomes from peripheral blood	Day of re-appearance	Remarks
1	40	0·33	1	5	...
2	Swarming	0·67	2	3	...
3	Swarming	0·68	1	2	...
4	Swarming	0·7	1	...	No relapse after 7 months
5	36	0·75	1	...	No relapse after 7 months
6	14	0·82	1	...	No relapse after 7 months
7	25	0·91	1	...	No relapse after 7 months
8	Swarming	1·2	...	...	Died in 22 hours. No. trypanosomes
Control	Swarming	0·17 (atoxyl)	1	17	...

It will be seen from the above table that while the atoxyl control animal was rendered free from trypanosomes in its peripheral blood

TABLE III.

Showing effect on mice infected with *T. rhodesiense.*

Experiment	Trypanosomes per field. Ocular 4, Obj. $\frac{1}{8}$	Dose in gms., per kilo	Day of disappearance of trypanosomes from peripheral blood	Day of re-appearance	Remarks
1	Swarming	0·5	2	4	...
2	Swarming	0·6	2	6	...
3	Swarming	0·74	2	5	...
4	Numerous	0·83	1	8	...
5	Numerous	0·9	2	5	...
6	Swarming	0·9	1	4	...
7	Numerous	1·0	2	5	...
8	Swarming	1·1	2	...	Alive and well 5 months later
9	Numerous	1·25	2	4	...
10	Numerous	1·6	1	6	...
11	Numerous	2·0	1	12	...
12	Swarming	2·5	2	7	...
13	Control	3·0	...	...	Died after 3 days

by 0·17 gms. atoxyl per kilo, but relapsed in seventeen days, the experimental animals were rendered free from trypanosomes when a dose of 0·7 gms. per kilo of phenylglycine amido arsenate of sodium was reached, and did not relapse.

Action in Vitro.

No trypanocidal action in vitro was observed, either by the drug itself or by the serum of animals, twenty-four hours after they had been rendered free from trypanosomes by injection of the drug.

Mice. Minimum Lethal Dose.

In Table III are set out the experiments performed in order to demonstrate the minimum lethal dose for mice and the effect on *T. rhodesiense.*

It will be seen from the table that the minimum lethal dose was 3 gms. per kilo; the effect on the trypanosomes was negligible up to a dose of 2·5 gms. per kilo, only one animal failing to relapse.

SUMMARY

Phenylglycine amido arsenate of sodium can be used in freshly prepared solutions in distilled water for intraperitoneal injection into rats and mice; the solutions on standing become toxic, and later become yellow in colour.

For rats, the minimum lethal dose of the freshly prepared drug proved to be 1·2 gm. per kilo of body weight.

For rats infected with *T. brucei*, the minimum curative dose is 0·7 gm. per kilo of body weight.

In vitro the drug has no appreciable action on trypanosomes, nor has the blood of treated animals immediately (twenty-four hours) after becoming trypanosome-free.

The drug has no curative effect on mice infected with *T. rhodesiense.*

A remarkable feature of this drug is its relatively high minimum lethal dose. Although the drug contains 26 per cent. arsenic, the minimum lethal dose was found to be 1·2 gms. per kilo for rats and 3 gms. for mice.

I am indebted to Professor Blacklock for carrying out the necessary inoculations for the experiments.